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PATENT ABSTRACTS OF JAPAN

(11)Publication number:

2001-128679

(43) Date of publication of application: 15.05.2001

(51)Int.CI.

C12N 15/09

C12Q 1/04

C12Q 1/68

(21)Application number : 11-312525

(71)Applicant: MARINE BIOTECHNOL INST CO LTD

(22)Date of filing:

02.11.1999

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HARAYAMA SHIGEAKI

(54) METHOD FOR IDENTIFYING AND SPECIFICALLY DETECTING SLOWLY GROWING MYCOBACTERIA USING CHARACTERISTIC BASE SEQUENCE PRESENT IN DNA GYRASE GENE (57)Abstract:

PROBLEM TO BE SOLVED: To allow accurately identifying/detecting slowly growing mycobacteria which can not be easily detected so far.

SOLUTION: This is a method for identifying and detecting slowly growing mycobacteria utilizing a characteristic base sequence present in gyrB gene.

LEGAL STATUS

[Date of request for examination]

05.09.2000

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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(19)日本国特許庁 (JP)

(12) 公開特許公報(A)

(11)特許出願公開番号 特開2001-128679 (P2001-128679A)

(43)公開日 平成13年5月15日(2001.5.15)

(51) Int.Cl.7	識別配号	ΡI	テーマコード(参考)
C 1 2 N 15/09	ZNA	2.2.2.2.4.4.	, , , , , , , ,
C 1 2 IV 10/05	ZNA	C 1 2 Q 1/04	4 B 0 2 4
C 1 2 Q 1/04		1/00	
		1/68	A 4B063
1/68		C 1 2 N 15/00	ZNAA
			ZNAA

審査請求 有 請求項の数5 OL (全57頁)

(21)出願番号 特願平11-312525 (71)出願人 591001949 株式会社海洋バイオテクノロジー研究所 (22)出顧日 平成11年11月2日(1999.11.2) 東京都文京区本郷1丁目28番10号 (72)発明者 笠井 宏朗 岩手県釜石市平田第3地割75番1 株式会 社海洋パイオテクノロジー研究所釜石研究 所内 (72)発明者 江崎 孝行 岐阜県岐阜市司町40番地 岐阜大学医学部 内 (74)代理人 100091096 弁理士 平木 祐輔 (外2名)

最終頁に続く

(54) 【発明の名称】 DNAジャイレース遺伝子中に存在する特徴的な塩基配列を用いた遅発育性マイコパクテリアの 同定法及び特異的検出法

(57)【要約】

【解決手段】 gyrt遺伝子中に存在する特徴的な塩基配列を利用して遅発育性マイコバクテリアの同定及び検出を行う方法。

【効果】 従来、同定及び検出が困難であった遅発育性マイコバクテリアについても正確に同定・検出を行うことが可能になる。

【特許請求の範囲】

【請求項1】 遅発育性マイコバクテリアのDNAジャイ レースβサブユニットをコードするDNA中の配列番号7. ~22に対応する領域を増幅し、その増幅断片の塩基配 列を配列番号7~22記載の塩基配列と比較し、各配列 からの遺伝学的距離を求め、その遺伝学的距離により前 記遅発育性マイコバクテリアの同定を行うことを特徴と する遅発育性マイコバクテリアの同定方法。

【請求項2】 配列番号4記載のアミノ酸配列の一部若 しくは全部をコードする配列、又はそれと相補的な配列 を含み、プライマー又はプローブとして実質的に機能す るオリゴヌクレオチドを、プライマー又はプローブとし で用いることにより。マイコバクテリウム・カンサシイ |を検出することを特徴とするマイコバクテリウム・カン サシイの検出方法。

《請求項3】 配列番号4記載のアミノ酸配列の一部若 しくは全部をコードする配列、又はそれと相補的な配列 を含み、プライマー又はプローブとして実質的に機能す るオリゴヌクレオチドを含むことを特徴とするマイコバ クテリウム・カンサシイの検出用キット。

【請求項4】 配列番号6記載のアミノ酸配列の一部若 しくは全部をコードする配列、又はそれと相補的な配列 を含み、プライマー又はプローブとして実質低に機能す るオリゴヌクレオチドを、プライマー又はプローブとし て用いることにより、マイコバクテリウム・ガストリを 検出することを特徴とするマイコバクテリウム・ガスト - リの検出方法。

【請求項5】 配列番号6記載のアミノ酸配列の一部若 しくは全部をコードする配列、又はそれと相補的な配列 を含み、プライマー又はプローブとして実質低に機能す るオリゴヌクレオチドを含むことを特徴とするマイコバ クテリウム・ガストリの検出用キット。

【発明の詳細な説明】

[0001]

【発明の属する技術分野】本発明は、結核並びに非結核 性抗酸症の原因菌として多くの症例数のある遅発育性マ イコバクテリアのDNAジャイレースβサブユニットをコ ードするDNA(以下、「gyrB遺伝子」という)の塩基配列 を利用した同定・検出法に関するものである。本発明の 同定・検出法は、各種産業分野において有用である。

[0002]

【従来の技術】ヒトに結核並びに結核類似症を引き起こ す抗酸菌種は遅発育性マイコバクテリアの属する種が複 数知られている。その中でも臨床例としてはマイコバク テリウム・ツベルクローシス・コンブレックス (Mycoba cterium tuberculosis complex)、マイコバクテリウム ・アビウム・コンプレックス (Mycobacterium avium co mplex)、マイコパクテリウム・カンサシイ (Mycobacte rium kansasii) がその大半を占めている。最近では後

細菌が全身播種性感染を引き起とし、AIDS患者の予後の 重大な問題となっている。

【0003】従来とれらの菌種の同定・検出は、培養に 基づく生理生化学的な方法によって行われていた。例え ば、遅発育性マイコバクテリアの中には、1)光を照射 した後に暗所で培養した場合にのみ黄色に発色するグル ープ(光発色菌)、2)光を照射せずに培養しても発色 するグループ(暗発色菌)、3)光を照射しても発色し ないグループ (非発色菌) の3 グループが存在するの で、この発色の違いを利用して同定・検出を行うことが 行われてきた。また、培養した細菌がカタラーゼ生産を 行うかどうか、ウレアーゼ活性、トゥイーン加水分解活 性あるいは硝酸塩還元活性を示すかどうか、などにより 同定・検出を行う方法も知られていた。

【0004】しかし、これらの検査は純粋培養を必要と する上、比較の対象となる表現型は変化しやすく、判定 が主観的になりがちであった。その結果、時間がかかる 上に、正確な種の判定は極めて困難であった。この様な 問題点を解決するために、近年になってポリメラーゼ連 鎖反応(以下、「PCR」という)法などを用いて遺伝子 の特定の塩基配列の有無を判定する同定・検出の方法が 考案され利用されるようになった。PCR法は、培養を必 要とせず迅速かつ客観的な判定を得るという点で、遅発 育性のマイコバクテリアの同定・検出に適している。 【0005】その際、用いられる遺伝子は多くの場合、 rRNA遺伝子である。T. Rogallら(1990. J. Gen. Micro biol. 136, 1915-1920) は、16S rRNA配列を用いたPCR に基づくマイコバクテリア種の同定法を提案した。しか しながら、これらのブライマーは、異なった表現型特性 を示すマイコバクテリウム・ガストリ(Mycobacterium gastri) とマイコバクテリウム・カンサシイを区別でき なかった。一方B. Boddinghausら(1990. J. Clin. Mic robiol. 28: 1751-1759) は、ヒト型結核菌グループや 鳥型結核菌-パラ結核菌およびマイコパクテリウム・イ ントラセルラーレ (Mycobacterium intracellulare) グ ループに特異的である16S rRNA配列に由来するオリゴヌ クレオチドを報告した。このオリゴヌクレオチドを用い ても種のレベルでの同定を行う為に必要な解像度は得ら れなかった。これらのrRNA遺伝子配列を利用した同定法 40 は、現在商品化され、日本ロッシュから「アンプリコア ・マイコバクテリウム」という商品名の遺伝子診断キッ トとして販売されている。その他にも、東洋紡社 (特開 平10-323189号公報) やベクトン・ディッキンソン・ア ンド・カンパニー(特開平10-057098号公報)からrRNA 配列を利用した検出あるいは同定法が開示されている。 上に記したような二つの種を判別できない問題を解決す るために、165 rRNAと235 rRNAの間の領域の配列を用い た同定・検出法がA. Rothらによって提案されている(1 998. J. Clin. Microbiol. 36: 139-147)。しかし、16 天性免疫不全症候群(AIDS)患者等においてはこれらの 50 S rRNAと23S rRNAの間の領域は約200塩基対しかなく、

とのような短い配列により精度の高い分子系統学的解析 は困難で二菌種のどちらの配列とも一致しない中間的な 配列を持つ新規株が現れた場合、どちらにより近縁かと いった判断を下すことができない一方、シュードモナス (Pseudomonas) 属、アシネトバクター (Acinetobacte r) 属を始め、多くの細菌で、進化速度の速いタンパク 質をコードする遺伝子、なかでもgyrB遺伝子の1200塩基 対の配列を用いることによって、より詳細で正確な分類 ・同定ができることが示された (Yamamoto, S. and S. -1109. Yamamoto, S. and S. Harayama. 1996. Int. J. Syst. Bacteriol. 46: 506-511. Harayama, S. and S. Yamamoto. 1996. p250-258 In Molecular Biology of Pseudomonas T. Nakazawa, K. Fukuda, D. Haas, S. Si Iver (eds) ASM press, Washington, D.C., 山本 敏、 原山重明、化学と生物 1996 第34巻 第3号 p. 149-15 1. 山本 敏、原山重明、農芸化学会誌 1997 第71巻 第9号 p.894-897.)。

【0006】gyrB遺伝子以外のタンパク質をコードする 遺伝子を用いて遅発育性マイコバクテリアの同定を行う 試みは行われている。例えば、C. T. Shivannvarらはス ーバーオキシド・ディスムターゼ遺伝子を利用して遅発 育性のマイコバクテリアの系統関係と抗原性の関係を論 じ (1994. J. Clin. Microbiol. 32: 2801-2812)、D. S. Swansonらは65 kD 熱ショックタンパク質遺伝子を用 いて鳥型結核菌-パラ結核菌およびマイコバクテリウム ・イントラセルラーレグループの詳細な分類を試みた (1997. Int. J. Syst. Bacteriol. 47: 414-419)。米 国アボット・ラボラトリーズは特表平10-500567 (国際 公開番号W095/31571) としてrRNA遺伝子以外にもマイコ 30 バクテリウム・ツベルクローシスのタンパク質抗原Bを コードする遺伝子や、マイコパクテリウム・ツベルクロ ーシスの65 kD熱ショックタンパク質、10kD熱ショック タンパク質等の遺伝子配列並びに挿入配列IS987やIS611 の関連する配列を用いた検出法を開示している。 他に もベクトン・ディッキンソン・アンド・カンパニーはマ イコバクテリウム・パラツベルクロシスの70kg熱ショッ クタンパク質をコードする遺伝子に由来する検出・同定 するプローブを特開平06-319560として開示している。 しかしながら、これらの遺伝子のうち、分子系統学的に 40 得られるデーターと従来の分類学的な手法による種の同 定と比較した場合、矛盾が認められないことが示されて いるのはgyrB遺伝子のみであり(Yamamoto and Harayam a 1998. Int. J. Syst. Bacteriol. 48: 813-819. Yama moto et.al. 1999. Int. J. Syst. Bacteriol. 49: 87-95. Suzuki et al. 1999. Int.J. Syst. Bacteriol. in press, Kasai et al. 1999. Int. J. Syst. Bacterio 1. in press)、新規に分離された株についても正確な 分子系統学的な位置を決定でき、更に他の遺伝子では区 別できなかった近縁種の区別できることなどから他の遺 50 テリウム・カンサシイの検出方法、及び前記オリゴヌク

伝子を用いた方法より有効な方法である。

[0007]

【発明が解決しようとする課題】gyrB遺伝子を用いた細 菌の同定・検出法については、既に本出願人により出願 されている(特開平11-169175号公報)。しかし、この 公報中では、遅発育性マイコバクテリアの同定・検出を 行うに当たってgyrB遺伝子中のどの領域を利用すればよ いかについては明らかにされていなかった。

)

【0008】遅発育性マイコバクテリアには結核などの Harayama, 1995. Appl. Environ. Microbiol, 61: 1104 10 重要な疾病の原因細菌が含まれるため、この細菌群を正 確に同定・検出するための方法が強く望まれている。一 方、遅発育性マイコバクテリアは、生育速度が通常の細 菌よりも遅いため、細菌の培養を必須とする生理生化学 的方法では同定・検出が難しい。本発明は、このような 技術的背景の下になされたものであり、gyrB遺伝子を利 用した遅発育性マイコバクテリアの同定・検出方法を提 供することにある。

[0009]

【課題を解決するための手段】本発明者らは遅発育性マ 20 イコバクテリアの標準株のgyr8遺伝子配列を決定した。 それらの配列に対して臨床から分離された株の分類学的 な位置づけを行った。分類学的位置づけが矛盾のないと とを、細菌の種の同定の標準的方法であるDNA-DNA 交雑 法によって確認した。

【0010】また、遺伝子配列を利用した細菌の検出法 として最も一般的に用いられる16SrRNA遺伝子の配列で は区別できない非結核性抗酸菌マイコバクテリウム・ガ ストリとマイコバクテリウム・カンサシイの標準株のDN AからgyrB 断片をPCR法によって増幅し塩基配列を決定 した。得られた配列を比較したところ、両株の16S rRNA 遺伝子配列は同一であるにも関わらず、両株のgyr8遺伝 子配列1257塩基配列中66箇所が異なっていることを見い 出した(図1)。それらの配列の違いを利用して両菌種 それぞれに特異的なPCR増幅を可能にしたプライマーを 設計した。

【0011】本発明は、以上の知見により完成されたも のである。即ち、本発明は、遅発育性マイコバクテリア のgyrB中の配列番号7~22に対応する領域を増幅し、 その増幅断片の塩基配列を配列番号7~22記載の塩基 配列と比較し、各配列からの遺伝学的距離を求め、その 遺伝学的距離により前記遅発育性マイコバクテリアの同 定を行うことを特徴とする遅発育性マイコバクテリアの 同定方法である。

【0012】また、本発明は、配列番号4記載のアミノ 酸配列の一部若しくは全部をコードする配列、又はそれ と相補的な配列を含み、プライマー又はプローブとして 実質的に機能するオリゴヌクレオチドを、プライマー又 はプローブとして用いることにより、マイコバクテリウ ム・カンサシイを検出することを特徴とするマイコバク

レオチドを含むマイコバクテリウム・カンサシイの検出 用キットである。

【0013】更に、本発明は、配列番号6記載のアミノ 酸配列の一部若しくは全部をコードする配列、又はそれ と相補的な配列を含み、ブライマー又はブローブとして 実質低に機能するオリゴヌクレオチドを、ブライマー又 はプローブとして用いることにより、マイコバクテリウ ム・ガストリを検出することを特徴とするマイコバクテ リウム・ガストリの検出方法、及び前記オリゴヌクレオ

[0014]

【発明の実施の形態】以下、本発明を詳細に説明する。

(1)同定方法

本発明の遅発育性マイコバクテリアの同定方法は、遅発 育性マイコバクテリアのgyrB中の配列番号7~22に対* *応する領域をPCRにより増幅し、その増幅断片の塩基配 列を配列番号7~22記載の塩基配列と比較し、各配列 からの遺伝学的距離を求め、その遺伝学的距離により前 記遅発育性マイコバクテリアの同定を行うことを特徴と する。

【0015】本発明において同定とは、分子系統学的な 手法等によって細菌の分類学的な位置を定めることをい う。gyrB中の配列番号7~22に対応する領域を増幅す るためのブライマーとしては、例えば、配列番号39及び チドを含むマイコバクテリウム・ガストリの検出用キッ 10 配列番号40により表されるプライマーを例示することが できるが、とれらに限定されるわけではない。配列番号 - 7~22記載の塩基配列と、対応するアミノ酸配列及び 由来とする微生物の名称との関係は、下表の通りであ

[0016]

【表1]

	- 1 - 2 m2/1 m - 7 1 - 0	4.4に対 *
塩基配列	アミノ酸配列	由来とする微生物の名称
配列番号7	配列番号23	マイコバクテリウム・シミエ
配列番号8	配列番号24	コイコペクテリム・シミエ
配列番号9	配列番号25	マイコパクテリウム・ボビス
配列番号10	配列番号26	マイコバクテリウム・スツルガイ
配列番号11	配列番号27	マイコバクテリウム・マルモエンセ
配列番号12		マイコバクテリウム・イントラセルラー・
配列番号13	配列番号28	マイコハクテリウム・アピウム
配列番号13	配列番号29	マイコバクテリウム・ゴルドナナ
	配列番号30	アミコバクテリウム・アフリカヌム
配列番号15	配列番号31	マイコパクテリウム・ツベルクローシス
配列番号16	1 m2/11m3 /3 /2 /	マイコバクテリウム・ガストリ
配列番号17	配列番号3-3	マイコバクテリウム・マリヌム
配列番号18	配列番号34	マイコバクテリウム・ミクロティ
配列番号19		マイコバクラリカ・ミクロティ
配列番号20		マイコバクテリウム・アジアティカム
配列番号21		マイコパクテリウム・スクロファラセウム
		マイコハクアリウム・プランデル
<u> </u>	HILL 2 9 9 1.	マイコバクテリウム・パラツベルクロシス

【0017】遺伝学的距離は、例えば、Phylip プログ ラムの説明書にあるFelsenstein記載の方法に従って求 めることができる(Felsenstein, J. 1993. PHYLIP(Phy logenyInference Package)version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle, U.S.A.) .

【0018】(2)特異的検出

本発明のマイコバクテリウム・カンサシイの検出方法 は、配列番号4記載のアミノ酸配列の一部若しくは全部 をコードする配列、又はそれと相補的な配列を含み、プ ライマー又はプローブとして実質的に機能するオリゴヌ クレオチドを、プライマー又はブローブとして用いると とを特徴とするものである。また、本発明のマイコバク テリウム・カンサシイの検出用キットは、前記オリゴヌ クレオチドを含むことを特徴とするものである。

【0019】本発明のマイコバクテリウム・ガストリの

くは全部をコードする配列、又はそれと相補的な配列を 含み、プライマー又はプローブとして実質低に機能する オリゴヌクレオチドを、ブライマー又はプローブとして 用いるととを特徴とするものである。また、本発明のマ コバクテリウム・ガストリの検出用キットは、前記オリ ゴヌクレオチドを含むことを特徴とするものである。

【0.020】ととで、「プライマー又はプローブとして 実質的に機能する」とは、特異的なアニール又はハイブ リダイズが可能な程度の長さを有するという意味であ り、検出対象とするDNAとアニール又はハイブリダイズ する配列を有しているが、その長さが短いため非特異的 なアニール又はハイブリダイゼーションを頻繁に起こ し、特異的な検出に使用できないようなオリゴヌクレオ チドを排除する趣旨である。

【0021】マイコバクテリウム・カンサシイの検出に 利用できるオリゴヌクレオチドとしては、配列番号3に 検出方法は、配列番号6記載のアミノ酸配列の一部若し 50 より表されるオリゴヌクレオチドを例示でき、マイコバ

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クテリウム・ガストリの検出に利用できるオリゴヌクレオチドとしては、配列番号5により表されるオリゴヌクレオチドを例示できるが、これらに限定されるわけではない。

【0022】検出の対象とするDNAの調製、プライマーの作製及びそれを用いたPCR、プローブの調製及びそれを用いたハイブリダイゼーションは、常法に従って行うことができ、特別な方法を用いる必要はない。

【0023】なお、PCRに使用するプライマーは、両方が特異的にアニールするものである必要は必ずしもなく、一方は非特異的にアニールするものであってもよい。このような非特異的にアニールするプライマーの一例としては、配列番号1記載の塩基配列により表されるプライマーを挙げることができる。

[0024]

【実施例】〔実施例1〕配列番号39及び配列番号40記載の塩基配列により表されるオリゴヌクレオチドを利用して、臨床患者より分離された抗酸菌株8菌株(KPM 2201 T、KPM 2202、KPM 2203、KPM 2013、KPM 2014、KPM 198 8-5、KPM 2209、KPM 2212)のgyrt遺伝子配列を決定した。得られたgyrt配列と遅発育性マイコバクテリア同定用gyrt配列セット(配列番号7-38)を利用して分子系統学的解析により系統関係を推定した。分子系統学的解析は、分子系統学的解析用汎用プログラムClustal W(Thompson,J. D., D. G. Higgins,and T. J. Gibson. 199 4. Clustal W:improving thesensitivity of progressive multiple sequence alignment through sequencewei*

* ghting, positions-specific gap penalties and weigh t matrix choice. Nucleic Acids Res. 22: 4673-468 0.)あるいはPhylip (Felsenstein, J. 1993. PHYLIP(Ph ylogeny Inference Package) version 3.5c. Distribute d by the author, Department of Genetics, Universit y of Washington, Seattle, U.S.A.) 等を用い、両プロ グラムの使用説明書に従い、以下のように行った。配列 番号39及び配列番号40記載の塩基配列により表されるオ リゴヌクレオチドを使用して得られたqyrB配列と配列番 10 号7-38の遅発育性マイコバクテリア同定用gyrB配列セッ トをClustal Wプログラムにより多重整列ファイルを作 成する。多重整列させる際に用いるパラメーターの一例 は"Gap Open Penalty: 15.00;Gap Extension Penalty: 6.66; DNA weight matrix: IUB; DNA transition weigh t:0.5"である。得られた多重整列はアミノ酸配列から得 られる多重整列ファイルと比較し、問題のある箇所は訂 正する。次に、多重整列ファイルをもとに各配列間の遺 伝学的距離を算出する。算出には、Phylipのdnadistプ ログラムを使用する。算出はKimura 2-parameter model に従って行う。 得られた遺伝学的距離から近隣結合法 により系統樹を作成する。系統樹の分岐の確からしさは bootstrap確率を計算すること等により検定する。 一 方、上記8菌株を165 rRNA遺伝子を利用した手法及び生 化学的手法によっても同定した。以上の結果を表2に示 す。

【0025】 【表2】

株名	生化学的試験	16S rRNA 遺伝子	DNA 相同性試験
KPM 2201T	M. gordonae	M. gordonae	M. gordonae
KPM 2202	M. gastori	M. gordonae	M. gordonae
KPM 2203	M. gastori	M. gordonae	M. gordonae
KPM 2013	M. scrofulcerum	M. gordonae	新種
KPM 2014	M. scrofulcerum	M. gordonae	新種
KPM 1988-5	M. scrofulcerum	M. gordonae	新種
KPM 2209	M. scrofulcerum	M. gordonae	新種
KPM 2212	データーなし	M. gordonae	新種

【0026】表が示すように、上記8菌株のうち、KPM 2201T、KPM 2202、KPM 2203の3菌株は、マイコバクテリウム・ゴルドナエに属するものと同定されたが、残りのKPM 2013、KPM 2014、KPM 1988-5、KPM 2209、KPM 22 40 12の5菌株は、マイコバクテリウム・ゴルドナエと近縁ではあるが別種(新種)であることが示唆された(図3)。この結果を、検証するためにDNA-DNA交雑試験(Ezaki, T., Hashimoto, Y., Takeuchi, T., Yamamoto, H., Shu-Lin Liu, Matsui, K., & Yabuuchi, E(1988). J. Clin. Microbiol. 26, 1708-1713. Ezaki, T., Hashimoto, Y., Takeuchi & Yabuuchi, E(1989). Int. J. Syst. Bacteriol. 39, 224-229)を行ったところ新種であることが支持された。この結果は、遅発育性マイコバクテリア同定用gyrB配列セットが既知の株のみならず新50

種の株に対しても信頼性の高い結果を与えることを示している。

【0027】〔実施例2〕マイコバクテリウム・カンサシイとマイコバクテリウム・ガストリのgyr8遺伝子の塩基配列を比較し(図1)、マイコバクテリウム・カンサシイのgyr8遺伝子に特異的にアニールするプライマー(配列番号3)とマイコバクテリウム・ガストリに特異的にアニールするプライマー(配列番号5)を作製した。また、両菌種のgyr8遺伝子にアニールするプライマー(配列番号1)も作製した。

【0028】 これらのプライマーを用いて、臨床患者より単離された株 KPM 1004, KPM 1007, KPM KY256, KPM KY761, KPM KY768, KPM 1988-1, KPM 3502, KPM 3503の 菌体破砕液をついて PCRを行った。 PCR法による増幅条件

* Y761, KPM KY768, KPM 1988-1は配列番号 l と3に示した

組み合わせでのみ増幅断片が観察されたので(表3)、

これらの株は マイコバクテリウム・カンサシイと同定

された。また、 KPM 3502, KPM 3503は配列番号 1 と5に

示した組み合わせでのみ増幅断片が観察されたので (表

3)、これらの株はマイコバクテリウム・ガストリ と

同定された。判定に用いた電気泳動像は図2に示すとお

りである。これらの同定結果はDNA-DNA ハイブリダイゼ

は次のとおりである。

[0029]

95 ℃ 10分 1サイクル

95 ℃ 1分、68 ℃ 1分30秒 30サイクル

72°C 10分 1サイクル

プライマー濃度 各1 μΜ

各 100 µM

Ampli Taq COLD"及び添付のPCR buffer Iを使用(米国 Perkin Elmer社)

【0030】増幅されたDNA 断片を電気泳動法により解 10 析したところ、KPM 1004, KPM 1007, KPM KY256, KPM K*

ーション法による同定結果と一致した。 [0031] 【表31

	M. kansasii	M. gastri
配列番号1 配列番号3	增幅可。	增幅不可。
配列番号 1 配列番号 5	增幅不可。	增幅可。

[0032]

【発明の効果】本発明は、これまで同定することが困難 であった遅発育性マイコバクテリアの分類同定を正確に 20 となる。 行うことを実現するものである。また非結核性抗酸菌の うち16S rRNA 遺伝子配列に基づく同定法では鑑別が困 ※

※難であった菌種、例えばマイコバクテリウム・カンサシ イとマイコパクテリウム・ガストリイの迅速同定が可能

[0033]

【配列表】

SEQUENCE LISTING

<110> MARINE BIOTECHNOLOGY INSTITUTE CO., LTD

<120> DNA GYIRESU IDENSHICHU NI SONZAISURU TOKUCHYOUTEKINA ENKIHAIRETSU WO MOCHIITA CHIHATSUIKUSEI MAIKOBAKUTERIA NO DOUTEIHOU OYOBI TOKUITEKI K

Enshutsuhou -- -

<130> P99-0373

<160> 40

<170> PatentIn Ver. 2.0

<210> 1

<211> 20

<212> DNA

<213> Artificial Sequence

<400> 1

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<210> 2

<211> 6

<212> PRT

<213> Artificial Sequence

<400> 2

Gly Val Ser Val Val Asn

<210> 3

<211> 20

<212> DNA

<213> Artificial Sequence

<400> 3

gac ctt gtg cgg ggc ggc gg

<210> 4

<211> 6

```
<212> PRT
```

<213> Artificial Sequence

<400> 4

Ala Ala Pro His Lys Val

<210> 5

<211> 20

<212> DNA

<213> Artificial Sequence

<400> 5

cac ctt gtg ggg ggc ggt ga

<210> 6

<211> 6

<21.2> PRT

<213> Artificial Sequence

<400> 6

Thr Ala Pro His Lys Val

<210> 7

<211> 1263

<21.2> DNA

<213> Mycobacterium simiae

<220>

<221> CDS

<222> (1)..(1263)

<400> 7

ggg gag aac agt ggc tac acc gtc agc ggc ggg ttg cac ggg gtc gga 48 Gly Glu Asn Ser Gly Tyr Thr Val Ser Gly Gly Leu His Gly Val Gly

5 10

gtg tcg gtg gtc aac gcc ctg tcc acc cgc ctg gaa gtc aac gtc aag 96 Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asn Val Lys

cgt gac ggc tat gag tgg ttc cag tac tac gac cgg gcg gtg ccc ggc 14 Arg Asp Gly Tyr Glu Trp Phe Gln Tyr Tyr Asp Arg Ala Val Pro Gly

35 40 4

50 55 60 ttc tgg gcc gat cct gag atc ttc gaa acc acc cag tac gac ttc gag 240 Phe Trp Ala Asp Pro Glu Ile Phe Glu Thr Thr Gln Tyr Asp Phe Glu

65 70 75 80

acg gtg gcg cgc cgg ttg cag gaa atg gcg ttc ctc aac aag ggc ctg 288
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
85 90 95

acc atc aac ctc acc gac gaa cgt gtc gag cag gac gag gtg gtc gat 336 Thr Ile Asn Leu Thr Asp Glu Arg Val Glu Gln Asp Glu Val Val Asp

100 105 110

gag gtg gtt agc gac acc gcc gag gcg ccg aag tca gcc gag gag cag 384 Glu Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Glu Gln

115 120 125

gcg gcc gaa tcg gcc aag ccg cac aag gtc aag cac cgc acg ttc cac 4 Ala Ala Glu Ser Ala Lys Pro His Lys Val Lys His Arg Thr Phe His

1	A

130			14
	135	140	
tac ccg ggt ggg ttg	ntg gat ttc gtc a	ag cac atc aat cgo	acc aaa 480
Tyr Pro Gly Gly Leu \	/al Asp Phe Val L	ys His Ile Asn Arg	Thr Lys
	L 5 0	155	160
aac ccg atc cag cag a	igo gto ato gao t	tc gac ggc aaa gga	acc ggg 528
Asn Pro Ile Gln Gln S	er Val Ile Asp P	ne Asp Gly Lys Gly	Thr Glv
165	1	70	175
cac gaa gtc gag atc g	cg atg cag tgg a	ac got got tat too	gan too Erc
His Glu Val Glu Ile A	la Met Gln Trp As	in Gly Gly Tyr Ser	Glu Son
180	185	190	ora bei
gtg cac acc ttc gcc a	ac acc atc aac ac	C Cat gag ggc ggc	300 535 634
Val His Thr Phe Ala As	on Thr Ile Asn Th	r His Glu Gly Gly	acc cac 624
195	200	205	inr Mis
gag gag ggc ttc cgc ac	IC GCO cto acc to	a ata ata ass	
Glu Glu Gly Phe Arg Se	er Ala Leu The So	g yeg yeg aac aag	tac gcc 672
210	215		lyr Ala
aaa gac aag aag ctg ct		220	
aaa gac aag aag ctg ct Lys Asp Lys Lys Lei Lo	u lus Asmilus As	ccc aac ctc acc	ggc gac 720
Lys Asp Lys Lys Leu Le 225 23	u g ras yeb ras yet		ly Asp
4.5		235	240
gac atc cga gaa ggg ct	y yee geg gtg ato	tcc gtg aag gtc c	icc gag 768
Asp Ile Arg Glu Gly Le			lla Glu
245	250		55
ccg cag ttc gag ggc cag	act aag acg aaa	ctc ggc aac acc g	ag gtc 816
Pro Gln Phe Glu Gly Glr	Thr Lys Thr Lys	Leu Gly Asn Thr G	lu Val
260	265	270	•
aag tog ttt gtc cag aaa	gtc tgt aac gaa	caa ctc act cac to	gg ttc 864
Lys ser File val Gin Lys	Val Cys Asn Glu	Gin Leu Thr His T	rp Phe
2/3	280	285	
gag gcg aac ccg tcg gaa	gct aaa acc gtt	gta aac aag gcg gt	tt tcg 912
Giu Ala Ash Pro Ser Glu	Ala Lys Thr Val	Val Asn Lys Ala Va	al Ser
290	295	300	
tcg gcc cag gcc cgc att	gcg gcg cgt aag	gcg cgg gag ttg gt	g cgg 960
Ser Ala Gln Ala Arg Ile	Ala Ala Arg Lys	Ala Arq Glu Leu Va] Arg
310		315	320
cgt aag agt gct acg gat	ttg ggt ggg ttg	CCO DOC aan tto oc	t ast 1000
Arg Lys Ser Ala Thr Asp	Leu Gly Gly Leu	Pro Gly Lys Leu Al	tgat 1008
325	330		
tgc cgc tcg acg gat ccg		335 To tat ata ata as	
Cys Arg Ser Thr Asp Pro	Arg Lvs Ser Glu i	All Two Val Val Ca	aget 1056
340	345		ı GIY
gat tee geg ggt ggg teg g		350	
Asp Ser Ala Gly Gly Ser	la lve Som Clu A	gr yar tog atg tto	cag 1104
355	360		e GIn
		365	
gcg atc ttg ccg ctg cgc c	nn aay all att a	ac gtc gaa aag gcc	cgc 1152
Ala Ile Leu Pro Leu Arg (Arg
-	275	380	
atc gat cgg gtg ctg aaa a	ar acc gaa gtc c	ag gcc atc atc acc	gcg 1200
The Asp Arg Val Leu Lys A			Ala
350	39		400
ctg ggc acc ggc atc cac g	ac qaa tto gac af	C acc ass cto cot	* 4246

.

Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr 410 415 cac aag atc gtg ttg 1263 His Lys Ile Val Leu 420 <210> 8 <211> 1257 <212> DNA <213> Mycobacterium bovis <220> <221> CDS <222> (1)..(1257) <400> 8 tcg gac gcg tat gcg ata tct ggt ggt ctg cac ggc gtc ggc gtg tcg Ser Asp Ala Tyr Ala Ile Ser Gly Gly Leu His Gly Val Gly Val Ser 10 gtg gtt aac gcg cta tcc acc cgg ctc gaa gtc gag atc aag cgc gac Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Glu Ile Lys Arg Asp ggg tac gag tgg tct cag gtt tat gag aag tcg gaa ccc ctg ggc ctc 144 Gly Tyr Glu Trp Ser Gln Val Tyr Glu Lys Ser Glu Pro Leu Gly Leu 40 aag caa ggg gcg ccg acc aag aag acg ggg tca acg gta cgg ttc tgg 192 Lys Gln Gly Ala Pro Thr Lys Lys Thr Gly Ser Thr Val Arg Phe Trp 55 gcc gac ccc gct gtt ttc gaa acc acg gaa tac gac ttc gaa acc gtc Ala Asp Pro Ala Val Phe Glu Thr Thr Glu Tyr Asp Phe Glu Thr Val 65 70 75 gcc cgc cgg ctg caa gag atg gcg ttc ctc aac aag ggg ctg acc atc Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu Thr Ile aac ctg acc gac gag agg gtg acc caa gac gag gtc gtc gac gaa gtg Asn Leu Thr Asp Glu Arg Val Thr Gln Asp Glu Val Val Asp Glu Val 100 105 110 gtc agc gac gtc gcc gag gcg ccg aag tcg gca agt gaa cgc gca gcc 384 Val Ser Asp Val Ala Glu Ala Pro Lys Ser Ala Ser Glu Arg Ala Ala 120 125 gaa tcc act gca ccg cac aaa gtt aag agc cgc acc ttt cac tat ccg 432 Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His Tyr Pro got goc ctg gtg gac ttc gtg aaa cac atc aac cgc acc aag aac gcg 480 Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys Asn Ala 155 att cat agc agc atc gtg gac ttt tcc ggc aag ggc acc ggg cac gag 528 Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu 170 gtg gag atc gcg atg caa tgg aac gcc ggg tat tcg gag tcg gtg cac 576 Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val His 185 acc ttc gcc aac acc atc aac acc cac gag ggc ggc acc cac gaa gag

Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu	
195 200 205	,
ggc ttc cgc agc gcg ctg acg tcg gtg gtg aac aag tac gcc aag gac	
Gly Phe Ard Ser Ala Lou The Ser Vel Vel 4	672
Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys Asp	1
220	
cgc aag cta ctg aag gac aag gac ccc aac ctc acc ggt gac gat atc	720
Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp Ile	
225 230 235 240	
cgg gaa ggc ctg gcc gct gtg atc tcg gtg aag gtc agc gaa ccg cag	768
Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln	,,,,
245 250 255	
ttc gag ggc cag acc aag acc aag ttg ggc aac acc gag gtc aaa tcg	
Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser	816
260	
200	
ttt gtg cag aag gtc tgt aat gaa cag ctg acc cac tgg ttt gaa gcc	864
Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala	
275 280 285	
aac ccc acc gac tog aaa gto gtt gtg aac aag got gtg too tog gog	912
Asn Pro Thr Asp Ser Lys Val Val Val Asn Lys Ala Val Ser Ser Ala	
290 295 300	
caa gcc cgt atc gcg gca cgt aag gca cga gag ttg gtg cgg cgt aag	060
Gin Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys	960
305 310 345	
323 320	
age gec ace gae ate ggt gga ttg eec gge aag etg gee gat tge egt	1008
Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg	•
325 330 335	
tcc acg gat ccg cgc aag tcc gaa ctg tat gtc gta gaa ggt gac tcg	1056
Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser	
340 345 350	
gcc ggc ggt tct gca aaa agc ggt cgc gat tcg atg ttc cag gcg ata	1104
Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile	
355 360 365	
ctt ccg ctg cgc ggc aag atc atc aat gtg gag aaa gcg cgc atc gac	4450
Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp	1152
270 595	
. 380	
cgg gtg cta aag aac acc gaa gtt cag gcg atc atc acg gcg ctg ggc	1200
Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gly	
385 390 395 400	
acc ggg atc cac gac gag ttc gat atc ggc aag ctg cgc tac cac aag	1248
Thr Gly Ile His Asp Glu Phe Asp Ile Gly Lys Leu Arg Tyr His Lys	
405 410 415	
atc gtg ctg	1257
Ile Val Leu	12.37
⊘10⊳ 9	
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220>	
⟨2?1> CDS	
<222> (1) (1263)	

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ClA Cla	Asn	Ser	Gly	Tyr	Asn	۷a۱	Ser	Gly	GΙγ	Leu	His	Gly	۷a٦	Gly	
1			5					10					15		

- yal Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asp Ile Lys

 20
 25
 30
- cgt gac ggc cac aag tgg tcg cag ttc tac aac aag gcc gtg ccg ggc 144
 Arg Asp Gly His Lys Trp Ser Gln Phe Tyr Asn Lys Ala Val Pro Gly
 35 40 45
- acg ctc aaa cag ggt gaa gcc act aag aaa acc gga acg aca att agg 192
 Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg
 50 55 60
- ttc tgg gcc gac ccg gac atc ttc gag acc acc gaa tac gac ttc gag 240
 Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
 65 70 75 80
- acc gtg gca cgc cgg ctg cag gaa atg gca ttc ctg aac aag ggc ttg 288
 Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
 85 90 95
- acc atc aac ctc acc gac gag cga gtt gcc cag gac gag gtt gtc gac 336
 Thr Ile Asn Leu Thr Asp Glu Arg Val Ala Gln Asp Glu Val Val Asp
 100 105 110
- gag gtc gtc agc gac acc gcc gag gca ccc aag tcc gcc gaa gaa aag 38 Glu Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Glu Lys 115 120 125
- gcg gcc gaa tcc aaa ggg ccg cat aag gtt aag cac cgc act ttc cat 4 Ala Ala Glu Ser Lys Gly Pro His Lys Val Lys His Arg Thr Phe His 130 135 140
- tac ccc ggc ggg ctg atc gac ttc gtc aag cac atc aac cgg acc aag
 Tyr Pro Gly Gly Leu Ile Asp Phe Val Lys His Ile Asn Arg Thr Lys
 145
 150
 150
 160
- ago cog ato cag cag agt gto gto gco tto gao ggo aag ggt gaa ggg 528 Ser Pro Ile Gln Gln Ser Val Val Ala Phe Asp Gly Lys Gly Glu Gly 165 170 175
- cac gag gtc gag atc gcg atg cag tgg aac ggc ggc tat tcg gag tcg 576 His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser 180 185 190
- gtg cac acc ttc gcc aac acc atc aac acc cac gag ggc ggc acc cac 624
 Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
 195 200 205
- gaa gaa ggg ttc cgc agc gca ctg aca tcg gtg gtg aac aag tac gcc 672 Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala 210 215 220
- aaa gac aag aag ctg ctc aag gag aag gac gcc aac ctc acc ggc gac 720 Lys Asp Lys Lys Leu Leu Lys Glu Lys Asp Ala Asn Leu Thr Gly Asp 225 230 235 240
- gac att cgc gag ggc ctg gcc gcg gtc atc tcg gtg aaa gtt gcc gaa 768 Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ala Glu 245 250 255
- ccg cag ttc gag ggc cag acc aag acc aaa ctg ggt aac acc gag gtc 816

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Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
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            275
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   gag gcc aac ccg tcg gaa gcc aaa acc gtc gtg aac aag gcg gtc tcg
   Glu Ala Asn Pro Ser Glu Ala Lys Thr Val Val Asn Lys Ala Val Ser
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   tcg gca cag gcg cgt atc gcc gcc cgc aag gca cga gag ttg gtg cgt
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   cgc aag agc gct acc gat ctc ggt ggg ctg ccc ggc aag ctg gcc gac
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   Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
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   Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
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 ctg ggt acc gga att cac gac gag ttc gac ctc gcc aaa ctg cgc tac
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 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Leu Ala Lys Leu Arg Tyr
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gtg tcg gtg gtc aac gcg ttg tcg acc cgg ctc gag gtg gat gtc gcc
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Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asp Val Ala
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                                25
cgc gac ggc tac atg tgg tca cag ttc tac gat cac gcc gag ccg gga
Arg Asp Gly Tyr Met Trp Ser Gln Phe Tyr Asp His Ala Glu Pro Gly
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Thr	Leu 50		G]n	Gly	ر Glu	Ala 55		Lys	Thr	Thr	GTy 60		Thr	· Ile	Arg	
ttc			gat	ccc	gac			gac	ı acc	acc			gac	ttc	gag	240
			Asp													- 10
65					70					75		.,,	, 04	, ,,,,	80	
		aca	cgc	coa			gaa	ato	וחכם			aac	220	aat		288
			Arg													200
			,-,	85		• • • • • • • • • • • • • • • • • • • •			90			7311	Lys	95	LEU	
acg	atc	aac	ctc	acc	gac	gag	cgg	gto	agt	gaa	gag	gag	gto	qtc	qac	336
			Leu													
			100					105					110			
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Tyr	Pro	G٦y	G٦y	Leu	۷a٦	Asp	Phe	Val	Lys	His	Ile	Asn	Arg	Thr	Lys	
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His	Glu	Val	Glu	Ile	ΑΊa	Met	Gln	Trp	Asn	Ala	Gly	Tyr	Ser	Glu	Ser	
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er	Ala	Gln	Ala .	Arg	Ile	Ala .	Ala	Arg	Lys	Ala	Arg	Glu	Leu	Val .	Arg	
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                                                                     1152
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Thr Leu Lys Gln Gly Glu Ala Thr Lys Arg Thr Gly Thr Thr Ile Arg
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ttc tog gcc gac ccc gac atc ttc gag acc acc gag tac gac ttc gag
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Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
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acc atc aac ctc acc gac gag cgg gtg agc aac gag gag gtc gtc gac
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Thr Ile Asn Leu Thr Asp Glu Arg Val Ser Asn Glu Glu Val Val Asp
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		-														
															a aag	384
Glu	Val	۷a٦	Ser	- Asp	Thi	· Ala	Asp	Ala	a Pro	Lys	Ser	· Ala	ı Glı	ı Gil	ı Lys	
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Ala			Ser	· Thr	· Ala			Lys	Va T	Lys			Thi	· Phe	e His	
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3ei	PIU	116	Gin	165		Tie	116	ASp			GIY	Lys	GIV		GTy	
cac	nan	ato	nan			ato		taa	170		~~	t 2 C	tee	175	tcc	F76
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Glu		Asn	Pro	Ala	Asp	Ala	Lys	۷a٦	۷a٦	Va1	Asn	Lys	Ala	Val	Ser	
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	Ala	Gin	Ala	Arg		Ala	Ala	Arg	Lys		Arg	Glu	Leu	Val	Arg	
305					310					31.5					320	
							ggc									1008
arg	Lys	Ser	Ala	1hr 325	Asp	Leu	GΊγ	Gly	330	Pro	Gly	Lys	Leu	A1a 335	Asp	
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Cys	Arg	Ser	Thr	Asp	Pro	Arg	Lys	Ser	Glu	Leu	Tyr	Val	Val	Glu	Gly	
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tac ccc ggc ggc ctg gtc gac ttc gtc aaa cac atc aat cgc acc aaa

Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys

aac ccc atc cac cag agc atc atc gat ttc ggt ggg aag ggc ccc ggc

Asn Pro Ile His Gln Ser Ile Ile Asp Phe Gly Gly Lys Gly Pro Gly

480

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His	Glu	≀ Va1	Glu	Ιle	Ala	Met	G]n	Trp) Asr	า (กิง	/ GTy	Tyr	Sei	r Glu	ı Ser	
			180					185	i				190)		
gto	cac	acc	ttc	gcc	aac	acc	ato	aac	acç	cac	gaq	ggc	gge	aco	cac	624
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gaq	gaq	ggc	ttc	cgc	ago	gcg	ctg	acc	tcc	gtg	gtc	aac	aaç	tac	gcc	672
Glu	Glu	Gly	Phe	Arg	Ser	Ala	Leu	Thr	Ser	· Val	Val	Asn	Lys	Tyr	· Ala	
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Asp	Ile	Arg	Glu	Gly	Leu	Ala	Ala	۷a٦	Пe	Ser	Val	Lys	Val	Ser	Glu	
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			Pro													
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Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asp Ile Lys	
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170 175	
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Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His	
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Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala	
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Asp	He	Arg	Glu			Ala	Ala	. Val			· Val	Lys	Val	Ala	Glu	
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		ctt														1152
AIA		Leu	Pro	Leu	Arq		Lys	He	He	Asn		Glu	Lys	Ala	Arg	
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Leu	Gly	Thr			НIS	Asp	Glu	Phe		He	Thr	Lys	Leu	Arg	Tyr	
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His	Lys			Leu												
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<220		_														
<221																
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Ser .	ASP	АІА	ıyr .		тıе	ser	GIY	GIY		HIS	Gly '	val (Gly		Ser	
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gtg	ycc	aac	gcg	cta	CCC .	acc	cgg	CTC	gaa	gtc	gag	atc :	aag	cgc	gac	96

143 M 3 A A A A A A A A A A A A A A A A A	38
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Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu Thr Ile	
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Asn Leu Thr Asp Glu Arg Val Thr Gln Asp Glu Val Val Asp Glu Val	
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Glu Ser Thr Ala Pro His Lys Val Lys Can Are The Review	432
Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His Tyr Pro	
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190	
acc ttc gcc aac acc atc aac acc cac gag ggc ggc acc cac gaa gag	624
Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu	
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Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser	
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Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln	
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Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser	
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Gin Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys	200
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Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg	
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Lys Gln Gly Ala Pro Thr Lys Lys Thr Gly Thr Thr Val Arg Phe Trp
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Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu Thr Ile
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Asn Leu Thr Asp Gln Arg Val Thr Gln Asp Glu Val Val Asp Glu Val
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Glu Phe Thr Ala Pro His Lys Val Lys Lys Arg Thr Phe His Tyr Pro
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ggt ggc ttg gtt gac ttc gtc aag cac atc aac cgc acc aag aac gcc
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IJу	G٦٠	/ Le	u V	a٦	Asp	Pho	e Va	il Ly	's Hi	s II	e As	n A	rg Th	ir Li	vs A	۱sn	Δla	
145						150					15			_	,		160	
atc	ca	ag	c a	gc	ato	gto	ga	c tt	c to	c go	a aa	ia. a	ag ac	c a	מר כ	·ac		
Ile	His	S Se	r Se	er	Ile	· Va	l As	p Ph	e Se	r G1	V Lv	's G	ly Th	r Gi	lv H	hic.	Glu	320
					165					17			,			.75	J.U	
gtg	qaç	at	c go	g	atg	caç	ı tg	q aa	t gc	c qq	c ta	t to	g ga	a to			cac	576
۷a٦	Glu	11	e A1	a	Met	Glr	Τŋ	o Asi	n Al	a G1	ν Τν	r Se	er Gl	u Se	r V	a]	Hic	370
			18						18		. ,			19		٠.		
acc	ttc	gc	c aa	ıc .	acc	ato	aa	aco	c ca	t ga	g gg	c qa	g ac			aa	gaa	624
Thr	Phe	Ala	a As	n '	Thr	Ile	Ası	Thi	r His	s GT	u GT	y GT	y Th	r Hi	s G	 1u	Glu	024
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GΊγ	Phe	Arg) Se	r A	41a	Leu	Thr	Ser	· Va	l Va	l Ası	ı Ly	 5 Tyı	r A1.	a Lv	/S	Asp	٥, ٤
	210						215					22			_,		р	
cgc	aaa	ctç	ct	c a	aaa	gac	aag	gac	ccc	aac	: cto	ac	c ggo	qa	c q a	ac	atc	720
Arg i	Lys	Leu	Le	u L	_ys	Asp	Lys	Asp	Pro	Asr	Leu	. The	r GTy	/ Ası	o As	g	Ile	
225						230					235			·			240	
gg (gaa	ggg	tt	g g	јсс	gcg	gtg	att	tcg	gto	aaa	gto	ago	gaa	a cc	g ·	cag	768
lrg (วไน	Gly	Lei	A L	lla.	Ala	Val	Ile	Ser	Va1	Lys	Val	Ser	Glu	ı Pr	o i	G]n	
				2	45					250					25	5		
tc ç	jag	ggc	cag	a a	CC .	aag	acg	aaa	cta	ggc	aac	acc	gag	gto	aa	q ·	tcg	816
he (ilu	Gly	G٦r	ı T	hr	Lys	Thr	Lys	Leu	Gly	Asn	Thr	Glu	۷a۱	Ly	s :	Ser	
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he V	al	G1n	Lys	V	al (Cys	Asn	Glu	Gìn	Leu	Thr	His	Trp	Phe	Glu	u A	\1a	
		275						280					285					
			-	:-		_ =			-				47 50 .				-	=
ac c	cc (act	gat	ac	rt a	aaa :	acc	att	otc	226	220							

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                                 25
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Phe Trp Ala Asp Ser Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
acg gtg gcg cgg cgc ctg cag gag atg gcg ttc ctc aac aag ggc ctg
                                                                288
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
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                                    90
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Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys
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															gac	96
vaı	vai	ASN	20		Ser	Inr	Arg	Leu 25		. Val	Glu	He	20 : 30		Asp	
ggo	tat	gag	tgg	tct	cag	gtt	tat	gag	aag	tcg	gaa	ccc	ctg	ggc	ctc	144
IJ	Tyr	Glu	Trp	Ser	Gln	Val	Tyr	Glu	Lys	Ser	Glu	Pro	Leu	GTy	Leu	
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HId	Ary	Arg	Leu	85	Giù	Mec	Ala	me	eu 90		Lys	GIY	Leu		Tie	
aac	cta	acc	asc		200	ata	200	C22			atc	atc	a 26	95	ata.	226
		Thr														336
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		Thr														
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Πe	His	Ser	Ser	Ile	۷a٦	Asp	Phe	Ser	Gly	Lys	Gly	Thr	Gly	His	Glu	
				165					170					175		
		atc														576
/aı	Giu	IJe		мет	Gin	Пф	ASN		Gly	iyr	Ser	Glu		Val	His	
	ttc	acc	180	366	3+ <i>c</i>	226	366	185					190			63.4
		gcc Ala														624
1111	riie	195	ASII	1111	116	ASII	200	1115	Giu	GIY	Giy	205	пі5	Giu	Giu	
ממכ	ttc	cgc	aoc	aca	cta	aca		ata	ata	aac	aan		acc	220	asc	672
		Arg														0/2
,	210	, 	50.			215	J.,	٠	· u ·	, 6,,	220	. ,.	ліц	Lys	Дþ	
:ac		cta	cta	aaq	gac		gac	ccc	aac	ctc		aat	gac	gat	atc	720
		Leu														, 20
25	-				230	-	•			235		,			240	
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		Gly														
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                                                      270
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  Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala
          275
  aac ccc acc gac tcg aaa gtc gtt gtg aac aag gct gtg tcc tcg gcg
 Asn Pro Thr Asp Ser Lys Val Val Val Asn Lys Ala Val Ser Ser Ala
                          295
 caa gcc cgt atc gcg gca cgt aag gca cga gag ttg gtg cgg cgt aag
 Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys
                     310
                                          315
 age gee ace gae ate ggt gga ttg eee gge aag etg gee gat tge egt
                                                                   1008
 Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg
                                      330
 tcc acg gat ccg cgc aag tcc gaa ctg tat gtc gta gaa ggt gac tcg
                                                                   1056
 Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser
             340
                                 345
 gcc ggc ggt tot gca aaa agc ggt cgc gat tog atg tto cag gcg ata
                                                                   1104
 Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile
         355
                             360
 ctt ccg ctg cgc ggc aag atc atc aat gtg gag aaa gcg cgc atc gac
 Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp
     370
                         375
cgg gtg cta aag aac acc gaa gtt cag gcg atc atc acg gcg ctg ggc
                                                                   1200
Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gly
                     390
                                         395
acc ggg atc cac gac gag ttc gat atc ggc aag ctg cgc tac cac aag 1248
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gtg tca gtg gtc aac gcg ttg tcg acc cga ctc gag gtc gac atc aag
Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asp Ile Lys
cgc gac ggg cac gag tgg tcc cag tat tac gag cgc gcc gtt cct ggc
                                                                  144
Arg Asp Gly His Glu Trp Ser Gln Tyr Tyr Glu Arg Ala Val Pro Gly
```

Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg

_	~	

	50)				55	i				60)				
tto	tgg	gco	gac	ccg	gac	ato	tto	gag	acc	acc	caç	tac	gad	tto	qaq	240
Phe	Trp	Ala	Asp	Pro	Asp	He	Phe	์ ไปน	Thr	Thr	G]n	Tyr	Asp	Phe	Glu	
65	i				70					75					80	
															ttg	288
Thr	Val	Ala	Arg	Arg	Leu	Gln	Glu	Met	Ala	Phe	Leu	Asn	Lys	Gly	Leu	
				85					90)				95		
			ttg													336
Thr	Ile	Asn	Leu	Thr	Asp	Glu	Arq	Val	Asp	Gln	Asp	Glu	۷a٦	Val	Asp	
			100					105					110			
			agc													384
Glu	۷a۱	Vaî	Ser	Asp	Thr	Ala	Asp	Ala	Pro	Lys	Ser	Ala	Glu	Glu	Lys	
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			tcc													432
Ala	Ala	Glu	Ser	Lys	Ala	Pro	His	Lys	Val	Lys	His	Arg	Thr	Phe	His	
	130					135					140					
			ggc													480
Tyr	Pro	Gly	Gly	Leu	Val	Asp	Phe	Va1	Lys	His	Ile	Asn	Arg	Thr	Lys	
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			caa													528
Ser	Pro	Ile	G]n	Gln	Ser	Val	Ile	Asp	Phe	Glu	Gly	Lys	Gly	Thr	Gly	
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			gag													576
His	Glu	Val	Glu	Ile	Ala	Met	Gln	Trp	Asn	IJу	GJy	Tyr	Ser	Glu	Ser	•
			180					185					190			
			ttc													624
Val	His		Phe	Ala	Asn	Thr	He	Asn	Thr	His	Glu	Gly	GJA	Thr	His	
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Glu		Gly	Phe	Arg	Ser	Ala	Leu	Thr	Ser	Val	Val	Asn	Lys	Tyr	Ala	
	210					215					220				•	
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	Asp	Lys	Lys	Leu	Leu	Lys	Asp	Lys	Asp	Pro	Asn	Leu	Thr	Gly	Asp	
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Asp	Пe	Arg	Glu		Leu	Ala	Ala	Val	Ile	Ser	Val	Lys	Val	Ala	Glu	
				245					250					255		
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Pro	Gln	Phe	Glu	Gly	GIn	Thr	Lys	Thr	Lys	Leu	Gly	Asn	Thr	Glu	Val	
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			qtq													864
Lys			Val	GIn	Lys	Vai	Cys	Asn	Glu	GIn	Leu	Thr	His	Trp	Phe	
		275					280					285				
			ccg													912
Glu		Asn	Pro	Ser			Lys	Thr	Val	Val	Asn	Lys	Ala	Val	Ser	
	290					295					300					
			gcc													960
Ser	Ala	Gln	Ala .	Arg	Ile .	Ala	Ala	Arg	Lys	Ala	Arg	Glu	Leu	Val	Arg	

305 310 315 cgc aag agc gcg acc gat ttg ggc ggg ctg ccc ggc aag ctg gcc gac 1008 Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp 325 tgc cgt tcc acc gac ccg cgc aag tcc gaa ctg tat gtg gtg gag ggt 1056 Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly 345 gac tcg gca ggt ggc tcg gcc aag agc ggc cgt gac tcg atg ttc cag 1104 Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln 355 gcc atc ctg ccg ctg cgc ggc aag atc atc aac gtc gag aag gcc cgc 1152 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg 375 atc gac cgg gtc ctg aag aac acc gaa gtc cag gcg atc atc acc gcg Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala 385 390 ctg ggt acc ggt att cac gac gag ttc gac att tct aaa ctg cgt tac 1248 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Ser Lys Leu Arg Tyr 410 cac aag atc gtg ttg 1263 His Lys Ile Val Leu 420 <210> 20 <211> 1263 <212> DNA <213> Mycobacterium scrofulceum <220> <221> CDS <222> (1)..(1263) <400> 20 ggc gag aac agc ggc tac acc gtc agc ggt ggg ttg cac gga gtg ggc Gly Glu Asn Ser Gly Tyr Thr Val Ser Gly Gly Leu His Gly Val Gly 1 5 gtg tcg gtg gtc aac gcg ctg tcc acc cgc ctg gag gtc acc atc aag Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Thr Ile Lys cgc gac ggg cac gag tgg ttt cag tac tac gac cgc gcc gtg ccc gga Arg Asp Gly His Glu Trp Phe Gln Tyr Tyr Asp Arg Ala Val Pro Gly 40 acc ctc aag cag ggc gag gcc acc aag aag acc gga acc acg atc agg 192 Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg ttc tgg gcg gac ccc gaa atc ttc gaa acc aca cag tac gac ttc gag 240 Phe Trp Ala Asp Pro Glu Ile Phe Glu Thr Thr Gln Tyr Asp Phe Glu acc gtg gcg cgg cgg ctg cag gag atg gcc ttc ctc aac aag ggc ctc 288 Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu acc atc aac ctc acc gac gaa cga gtg gag cag gac gag gtc gtc gac Thr Ile Asn Leu Thr Asp Glu Arg Val Glu Gln Asp Glu Val Val Asp

			100					105					110	+		
aaa	atc	ato	agc	aac	acc	acc	aaa	aca	cca	aaa	tcc	acc			aaq	384
			Ser									•	• ·			501
		115		. – ۴		,,,,	120			_,_	٥.,	125			-,5	
acc	aca		tcg	act	oca	cca			atc	aad	cac			ttc	cac	432
			Ser													7,72
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tac		aac	ggt	cta	atc		ttc	atc	224	cac			cac	366	220	480
			GTy													460
145	110	diy	Giy	Leu	150	изр	riie	vai	Lys	155	116	ASII	Arg	1111		
	cca	atc	cag	C20		atc	ate	ast	++c		~~	220	225	200	160	E20
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<i>3</i> e1	FIU	116	Gln		Ser	Vai	Tie	ASP		ASP	Gly	Lys	GIγ		GIY	
636	000	atc		165		240		+	170			.	.	175		
			gag													576
HIS	Giu	vai	Glu	Tie	АІА	мет	GIN		ASN	GIY	Gly	lyr		Glu	Ser	
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			ttc													624
vai	HIS		Phe	Ala	Asn	Ihr		Asn	Thr	His	Glu		Gly	Thr	His	
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Glu		Gly	Phe	Arg	Ser		Leu	Thr	Ser	Val	Val	Asn	Lys	Tyr	Ala	
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Lys	Asp	Lys	Lys	Leu	Leu	Lys	Asp	Lys	Asp	Pro	Asn	Leu	Thr	Gly	Asp	
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Asp	He	Arg	Glu	GΊγ	Leu	Ala	Ala	۷a٦	IJе	Ser	Val	Lys	Val	Ala	ดีใน	
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cca	cag	ttc	gaa	ggc	cag	acc	aag	aca	aag	ctg	ggc	aac	acc	gag	ata	816
Pro	Gln	Phe	Glu	Gly	GIn	Thr	Lys	Thr	Lys	Leu	Gly	Asn	Thr	Glu	Val	
			260					265					270			
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Lys	Ser	Phe	Val	Gln	Lys	Val	Cys	Asn	Glu	Gln	Leu	Thr	His	Trp	Phe	
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gag	gcc	aac	cca	tcc	gag	gcg	aaa	acg	gtg	gtg	aac	aaa	gcg	gtg	tcg	912
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tcq	qct	caq	gcg	cqc	att	qcc	qcc	cqc	aaq	qcq	cat	qaa	cta	ata	cac	960
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	aaa	agc	gcc	acc		ctc	aac	aat	cta		aaa	aan	cta	ncc		1008
			Ala													,
,-,	-,-	.		325	, Op		J.,	٠.,	330		U17	LyJ	LCU	335	љр	
tac	coc	tcc	acc		רכח	con	222	tca		cta	tat	ata	ata		ooc	1056
																1030
-y3	n y	JC1	Thr 340	√⊃h	110	nı y	LYS		JIU	ren	ı yr	vai		JIU	JIY	
na t	tca	occ		aac	tcc	occ	227	345	000	ccc	a 26	tca	350	++-	c>=	1104
			ggc													1104
۳ъþ			Gly	uιγ	ær	AId		ser	uly	Arg	ASP		met	me	UIN	
		355					360					365				

1263

144

gcg atc ctg ccg ctg cgc ggc aag atc atc aat gtc gag aag gcc cgc 1152 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg 370 375 380

atc gac cgg gtg ctg aag aac acc gaa gtt cag gcg atc atc acc gcg

Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala

385

390

395

400

ctg ggt acc ggg att cac gac gag ttc gac atc acc aag ctg cgc tat 1248 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr

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vai Leu 420

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acc gac ggg tac gag tgg ttt cag cat tac gac cgc tct gtc ccc ggc
Thr Asp Gly Tyr Glu Trp Phe Gln His Tyr Asp Arg Ser Val Pro Gly
35 40 45

ttc tog gcc gac ccg gac atc ttc gag acg acg gat tac gac ttc gag
Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Asp Tyr Asp Phe Glu
65 70 75

acg gtc gca cgc cgg ctg cag gaa atg gcg ttc ctc aac aaa ggg ctg 288
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
85

acc atc aac ctg acc gac gag cgg gtg cga aac gaa gaa gtc gtc gac 336
Thr Ile Asn Leu Thr Asp Glu Arg Val Arg Asn Glu Glu Val Val Asp
100 105 110

gag gtc gtc agc gac acc gcc gac gcg ccg aag tcg gcg cgc gaa gag 384 Glu Val Val Ser Asp Thr Ala Asp Ala Pro Lys Ser Ala Arg Glu Glu

9cc gaa gaa cgg acc acg cag aaa gtc aag cac cgc acg ttc cat tac 432
Ala Glu Glu Arg Thr Thr Gln Lys Val Lys His Arg Thr Phe His Tyr
130 135 140

ccc ggc ggc ttg gtc gat ttc gtc aaa cac atc aac cgc aca aag aac 480

6	1	

	,)4
		GTy	/ Lei	ı Val	Asp	Phe	· Va	Lys	His	i Ile	Asn	Arç	Thi	r Lys	s Asn	
145					150					155					160	
															cac	528
ric	, 116	. 1115	ser	165		. vai	ASP	me	5er 170		Lys	GIY	/ Pro		/ His	
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															val	576
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His	Thr	Phe	Ala	Asn	Thr	Ile	Asn	Thr	His	ตน	Gly	Gly	Thr	His	Glu	
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Glu			Arg	Ala	Ala		Thr	Ser	Val	۷a٦	Asn	Lys	Tyr	Ala	Lys	
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								gaa								864
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								gtc Val								912
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.ys	Ser	Ala	Thr	Asp	Leu	G٦y	Gly	Leu	Pro	Gly	Lys	Leu	Ala	Asp	Cys	
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								gaa								1056
\rg	Ser	Thr		Pro	Arg	Lys	Ser	Glu	Leu	Tyr	Val	Val		Gly	Asp	
	255		340	+ c=				345					350			
								ggc								1104
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tc	cta		tta	caa	aac	aan		atc	aac	ata	nan	365 aad	acc	cac	atc	1152
								Ile								1172
-	370	-		·· :=#	,	375				•	380	_,_		9		
ac	cgg	gtg	ctg	aag	aac		gag	gtg	cag	gcg		atc	acc	gcq	ctg	1200
								Val								
85					390					395					400	
qс	acc	ggg	att	cac	gac	gag	ttc	gac	atc	tcc	aag	ctg	cgc	tac	cac	1248
ηy	Thr	Gly	Πe	His	Asp	Glu	Phe	Asp	Ile	Ser	Lvs	Leu	Ara	Tvr	His	

410 415

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405

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35

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Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
85 90

acc atc aac ctc acc gac gag cgg gtg acc aac gaa gag gtc gtc gac 336
Thr Ile Asn Leu Thr Asp Glu Arg Val Thr Asn Glu Glu Val Val Asp
100 105 110

gag gtg gtc agc gac acc gcc gac gca ccc aag tcg gcg cag gag aag
Glu Val Val Ser Asp Thr Ala Asp Ala Pro Lys Ser Ala Gln Glu Lys
115
120
125

gcg gcg gaa tcg gct gcg ccg cat aag gtc aag cac cgc acc ttc cac 432 Ala Ala Glu Ser Ala Ala Pro His Lys Val Lys His Arg Thr Phe His

tac ccc ggc ggc ctg gtc gac ttc gtc aaa cac atc aat cgc acc aaa 480 Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys

145 150 155 160 aac ccc atc cac cag agc atc atc gat ttc ogt gog aag ggc ccc ogc 52

Asn Pro Ile His Gln Ser Ile Ile Asp Phe Gly Gly Lys Gly Pro Gly

165

170

175

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gtg cac acc ttc gcc aac acc atc aac acg cac gag ggc ggc acc cac 624 Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His

67

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Arg Asp Gly Tyr Glu 35	Trp Phe Gln Tyr Tyr A	sp Arg Ala Val Pro Gly
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50	55	hr Gly Thr Thr Ile Arg 60
Phe Trp Ala Asp Pro 65	70	hr Gln Tyr Asp Phe Glu
Thr Val Ala Arg Arg		75 80
85	Leu Gîn Gîu Met Ala Pi 90	95
inr Ile Asn Leu Thr	Asp Glu Arg Val Glu Gl	n Asp Glu Val Val Asp
100	105	110
Glu Val Val Ser Asp	Thr Ala Glu Ala Pro Ly	's Ser Ala Glu Glu Gln
115	120	125
Ala Ala Glu Ser Ala (ys Pro His Lys Val Ly	S His Ara The Dha Wis
130	135	140
Tyr Pro Gly Gly Leu V	al Asp Phe Val Lys Hi	S Ile Asn Ara The Lie
145 1	50 15	-
Asn Pro Ile Gln Gln S	er Val Ile Asp Phe Asp	
165	170	
His Glu Val Glu Ile A	la Met Gln Trp Asn Gly	175
180	185	
	103	190
Val His Thr Phe Ala Ad	on Thr Ile Asn Thr His	Glu Gly Gly Thr His
		205
Glu Glu Gly Phe Arg Se	er Ala Leu Thr Ser Val	Val Asn Lys Tyr Ala
210	215	220
Lys Asp Lys Lys Leu Le	u Lys Asp Lys Asp Pro	Asn Leu Thr Gly Asp
223 23	0 235	240
Asp Ile Arg Glu Gly Le	u Ala Ala Val Ile Ser	Val Lys Val Ala Glu
245	250	255
Pro Gin Phe Glu Gly Gl	n Thr Lys Thr Lys Leu	Gly Asn Thr Glu Val
260	265	270
Lys Ser Phe Val Gln Lys	S Val Cys Asn Glu Gln	Leu Thr His Tro Phe
275	280	285
Glu Ala Asn Pro Ser Glu	Ala Lys Thr Val Val	Asn Lys Ala Val Ser
290	295	300
Ser Ala Gln Ala Arg Ile	Ala Ala Arg Lys Ala	Ara Glu Leu Val Ara
305 310	315	320
Arg Lys Ser Ala Thr Asp	Leu Gly Gly Leu Pro	JZU GTV I VS I AU ATS Ass
325	330	335
Cys Arg Ser Thr Asp Pro		Sur Val Val Cluscos
340	345	
Asp Ser Ala Gly Gly Ser		350
355	360	
Ala Ile Leu Pro Leu Arg		365
370	375	80
Ile Asp Arg Val Leu Lys	Asn Thr Glu Val Gln A	la Ile Ile Thr Ala

395 400 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr

390

His Lys Ile Val Leu

420

<210> 24

<211> 419

385

<212> PRT

<213> Mycobacterium bovis

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Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Glu Ile Lys Arg Asp 25

Gly Tyr Glu Trp Ser Gln Val Tyr Glu Lys Ser Glu Pro Leu Gly Leu 40

Lys Gin Gly Ala Pro Thr Lys Lys Thr Gly Ser Thr Val Arg Phe Trp

Ala Asp Pro Ala Val Phe Glu Thr Thr Glu Tyr Asp Phe Glu Thr Val 70 75

Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu Thr Ile 90

Asn Leu Thr Asp Glu Arg Val Thr Gln Asp Glu Val Val Asp Glu Val 105

Val Ser Asp Val Ala Glu Ala Pro Lys Ser Ala Ser Glu Arg Ala Ala 120

Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His Tyr Pro

Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys Asn Ala

Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu 170

Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val His 185

Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu 200

Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys Asp

Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp Ile 230 235

Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln 250

Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser 265

Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala 280

Asn Pro Thr Asp Ser Lys Val Val Val Asn Lys Ala Val Ser Ser Ala 290 295 300

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Gìn Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys
                        310
                                            315
    Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg
                                        330
    Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser
                340
                                    345
    Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile
                                360
   Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp
                           375
   Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gly
                       390
                                           395
   Thr Gly Ile His Asp Glu Phe Asp Ile Gly Lys Leu Arg Tyr His Lys
                   405
                                       410
   Ile Val Leu
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   <211> 421
   <212> PRT
   <213> Mycobacterium szulgai
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                                   25
  Arg Asp Gly His Lys Trp Ser Gln Phe Tyr Asn Lys Ala Val Pro Gly
                               40
  Thr Leu Lys Gln Gly Glu-Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg
                           55
                                              60
  Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
                      70
                                          75
 Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
                                      90
 Thr Ile Asn Leu Thr Asp Glu Arg Val Ala Gln Asp Glu Val Val Asp
                                 105
 Glu Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Glu Lys
                             120
                                                 125
Ala Ala Glu Ser Lys Gly Pro His Lys Val Lys His Arg Thr Phe His
                         135
                                             140
Tyr Pro Gly Gly Leu Ile Asp Phe Val Lys His Ile Asn Arg Thr Lys
                    150
Ser Pro Ile Gln Gln Ser Val Val Ala Phe Asp Gly Lys Gly Glu Gly
                165
                                    170
His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser
Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
                            200
                                                205
Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala
                        215
Lys Asp Lys Lys Leu Leu Lys Glu Lys Asp Ala Asn Leu Thr Gly Asp
225
                    230
                                        235
                                                           240
```

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Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ala Glu
 Pro Gin Phe Glu Gly Gin Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
                                 265
Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
                            280
Glu Ala Asn Pro Ser Glu Ala Lys Thr Val Val Asn Lys Ala Val Ser
                        295
Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
                                        315
Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
                                    330
Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
                                345
Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
                            360
Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                        375
Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
                    390
                                        395
Leu Gly Thr Gly Ile His Asp Glu Phe Asp Leu Ala Lys Leu Arg Tyr
                                    410
His Lys Ile Val Leu
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<211> 421
<212> PRT
'<213> Mycobacterium malmoense
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Arg Asp Gly Tyr Met Trp Ser Gln Phe Tyr Asp His Ala Glu Pro Gly
Thr Leu Lys Gln Gly Glu Ala Thr Lys Thr Thr Gly Thr Thr Ile Arg
                         55
Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
                     70
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
                                     90
Thr Ile Asn Leu Thr Asp Glu Arg Val Ser Glu Glu Glu Val Val Asp
                               105
Asp Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Val Glu Lys
                           120
Ala Ala Glu Ser Thr Gly Pro His Lys Val Lys His Arg Thr Phe His
                       135
Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys
                    150
                                        155
Asn Pro Ile His Asn Ser Ile Val Asp Phe Ser Gly Lys Gly Pro Gly
```

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165
                                       170
    His Glu Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser
                                   185
   Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
                               200
   Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala
                           215
                                              220
   Lys Asp Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp
                      230
                                          235
   Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu
                                      250
   Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
                                  265
   Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
                              280
  Glu Ala Asn Pro Ala Asp Ala Lys Thr Val Val Asn Lys Ala Val Ser
                          295
  Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
                                         315
  Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
                 325
                                     330
  Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
                                 345
  Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
                             360
 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
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                                        395
 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr
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 His Lys Ile Val Leu
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 <212> PRT
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Arg Asp Gly Tyr Glu Trp Ser Gln Phe Tyr Asp His Ala Val Pro Gly
                             40
Thr Leu Lys Gln Gly Glu Ala Thr Lys Arg Thr Gly Thr Thr Ile Arg
Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
                    70
                                        75
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
                85
                                    90
```

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80
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Thr	Ile	Asn	Leu 100		Asp	Glu	Arg	Va1		Asn	Glu	GTu			Asp
СIII	Val	Val			Thr	α٦٥	Acn			Live	Son	۸٦-	110		Lys
0.0	vai	115		ΛSÞ	1711	Aid	120		rio	LYS	361	125		Giu	Lys
Ala	Ala			Thr	Αla	Pm	His		Val	lvs	His			Pho	Hic
	130					135		-,-		_,_	140		,		5
Tyr	Pro	Gly	Gly	Leu	Va1	Asp	Phe	Val	Lys	His	Ile	Asn	Arg	Thr	Lys
145					150					155					160
Ser	Pro	Пe	Gln	G٦n	Ser	Ile	Ile	Asp	Phe	Asp	۵٦y	Lys	G٦y	Pro	σīу
				165					170					175	
His	Glu	۷a٦	G٦u	Пe	Ala	Met	Gln	Trp	Asn	IJу	G٦y	Tyr	Ser	Glu	Ser
			180					185					190		
Val	His		Phe	Ala	Asn	Thr	Ile	Asn	Thr	His	Glu	Gly	Gly	Thr	His
~	63	195	_		_		200	_	_			205			
Giu		Gly	Phe	Arg	Ser		Leu	Thr	Ser	Val		Asn	Lys	Tyr	Ala
1.00	210	Lvc	Lvc	1	Lau	215	A	1.45	A	D	220	1	т	~ 7	• .
225	ASÞ	Lys	LyS	Leu	230	LyS	Asp	Lys	ASp	235	ASN	Leu	ınr	GIY	-
	Tle	Ara	Glu	Glv		Δla	Αla	Val	Πe		Va1	Lve	Val	Sar	240 Clu
		,		245		, <u></u>	,,,u	vu .	250	٠.,	Vai	LyJ	vai	255	diu
Pro	Gln	Phe	Glu		Gln	Thr	Lys	Thr		Leu	Gly	Asn	Thr		Val
			260					265			,		270		
Lys	Ser	Phe	Va1	Gln	Lys	Val	Cys	Asn	Glu	Gln	Leu	Thr	His	Trp	Phe
		275					280					285			
Cla	Ala	Asn	Pro	Ala	Asp	A٦a	Lys	Va1	Val	۷a٦	Asn	Lys	Ala	Val	Ser
	290					295					300				
	Ala	Gln	Ala	Arg		Ala	Ala	Arg	Lys		Arg	Glu	Leu	Val	Arg
305		c	47 -		310		~ 3	- 3		31.5					320
Arg	Lys	ser	АІА	1nr 325	ASP	Leu	Gly	Gly		Pro	Gly	Lys	Leu		Asp
Cvs	Δm	Ser	Thr		Pm	Δra	Lys	Sar	330 Clu	l ou	Tvr	Val	Val	335	αv
-,5	, ,,,	JC.	340	, Ор		A1 94	LyJ	345	Giu	LCU	1 41	Vai	350	Giu	diy
Asp	Ser	Ala	Gly	GTy	Ser	Ala	Lys		Gly	Ara	Asp	Ser		Phe	Gln
		355					360		·		•	365			
Ala	Пe	Leu	Pro	Leu	Arg	Gly	Lys	Пe	Ile	Asn	Val	GTu	Lys	Αla	Arg
	370					375					380				
Пe	Asp	Arg	Val	Leu	Lys	Asn	Thr	Glu	Val	Gln	Ala	Ile	Ile	Thr	Ala
385					390					395					400
Leu	Gly	Thr			His	Asp	Glu	Phe	Asp	Ile	Thr	Lys	Leu	Arg	Tyr
				405					410					415	
НIS	Lys		Val	Leu											
<210	⊳ 20		420												
<211															
<212															
			cter	ium	aviu	m									
<400															
Gly	Glu .	Asn	Ser	Gly	Tyr	Asn	۷a٦	Ser	Gly	Gly	Ĺeu	His	GΊγ	Val	Gly
1				5					10					15	
Val	Ser	Val	Val.	Asn	Ala	Leu	Ser	Thr	Arg	Leu	Glu '	Val	Asn	Ile	Ala

20	25	30
Arg Asp Gly Tyr Glu Trp S 35	er Gln Tyr Tyr / 40	Sp His Ala Val Pro Gly
		45
	\$5	60
Phe Trp Ala Asp Pro Asp I 65 70		75
Thr Val Ala Arg Arg Leu G		
85 Thr Ile Asn Leu Thr Asp G	90 lu Arg Val Thr A	95 sn Glu Glu Val Val Asp
100 Glu Val Val Ser Asp Thr Al	105	110
115	120	125
Ala Ala Glu Ser Ala Ala Pr	o His Lys Val Ly	s His Arg Thr Phe His
130 13	5	140
Tyr Pro Gly Gly Leu Val As	p Phe Val Lys Hi	s Ile Asn Arg Thr Lvs
145 150	15	55 160
Asn Pro Ile His Gln Ser Ile	e Ile Asp Phe Gl	y Gly Lys Gly Pro Gly
165	170	175
His Glu Val Glu Ile Ala Mei 180		y Gly Tyr Ser Glu Ser
	185	190
Val His Thr Phe Ala Asn Thr 195	Tie Asn Thr Hi: 200	s Glu Gly Gly Thr His 205
Glu Glu Gly Phe Arg Ser Ala		
210 215		220
Lys Asp Lys Lys Leu Leu Lys		
		ASII Leu Inr Giy Asp
Asp Ile Arg Glu Gly Leu Ala	Ala Val Tla Sor	C Val Tye Val Can (240)
245	250	
Pro Gin Phe Giu Giy Gin Thr		255 Gly Asn Thr Clu Vol
260	265	270
Lys Ser Phe Val Gln Lys Val		Leu Thr His Trn Dha
275	280	285
Glu Ala Asn Pro Ala Asp Ala	Lys Val Ile Val	Asn Lys Ala Val Ser
290 295		300
Ser Ala Gln Ala Arg Ile Ala	Ala Arg Lys Ala	Arg Glu Leu Val Arg
305 310	315	320
Arg Lys Ser Ala Thr Asp Leu	Gly Gly Leu Pro	Gly Lys Leu Ala Asp
325	330	335
Cys Arg Ser Thr Asp Pro Arg	Lys Ser Glu Leu	Tyr Val Val Glu Gly
340	345	350
Asp Ser Ala Gly Gly Ser Ala	Lys Ser Gly Arg	Asp Ser Met Phe Gln
355	360	365
Ala Ile Leu Pro Leu Arg Gly	Lys Ile Ile Asn	Val Glu Lys Ala Arg
370 375		380
Ile Asp Arg Val Leu Lys Asn 1		Ala Ile Ile Thr Ala
385 390	395	400
Leu Gly Thr Gly Ile His Asp (Thr Lys Leu Arg Tyr
405	410	415
His Lys Ile Val Leu		

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<211> 421

<212> PRT

<213> Mycobacterium gordonae

<400> 29

Gly Glu Asn Ser Gly Tyr Thr Val Ser Gly Gly Leu His Gly Val Gly

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Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asp Ile Lys
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Arg Asp Gly His Glu Trp Ser Gln Tyr Tyr Lys Arg Ala Val Pro Gly
35 40 45

Thr Leu Lys Gln Gly Glu Thr Thr Arg Lys Thr Gly Thr Thr Ile Arg

50 55 60

Phe Trp Ala Asp Pro Glu Ile Phe Glu Thr Thr Gln Tyr Asp Phe Glu
65 70 75 80

Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
85 90 95

Thr Ile Asn Leu Thr Asp Glu Arg Val Glu Gln Asp Glu Val Val Asp
100 105 110

Glu Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Glu Lys 115 120 125

Ala Ala Glu Ser Lys Ala Pro His Lys Val Lys Gln Arg Thr Phe His 130 135 140

Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys 145 150 155 160

Ser Pro Ile Gln Gln Ser Val Ile Asp Phe Glu Gly Lys Gly Thr Gly
165 170 175

His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser 180 185 190

Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glú Gly Gly Thr His 195 200 205

Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala 210 215 220

Lys Asp Lys Lys Leu Leu Lys Glu Lys Asp Pro Asn Leu Thr Gly Asp 225 230 235 240

Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ala Glu

Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
260 265 270

Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe 275 280 285

Glu Ala Asn Pro Ser Glu Ala Lys Thr Val Val Asn Lys Ala Val Ser 290 295 300

Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg 305 310 315 320

Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp 325 330 335

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Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
                                  345
   Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
                              360
   Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                          375
   Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
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  Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr
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                                     410
  His Lys Ile Val Leu
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 Gly Tyr Glu Trp Ser Gln Val Tyr Glu Lys Ser Glu Pro Leu Gly Leu
                              40
 Lys Gln Gly Ala Pro Thr Lys Lys Thr Gly Ser Thr Val Arg Phe Trp
      50
                         55
 Ala Asp Pro Ala Val Phe Glu Thr Thr Glu Tyr Asp Phe Glu Thr Val
  Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu Thr Ile
 Asn Leu Thr Asp Glu Arg Val Thr Gln Asp Glu Val Val Asp Glu Val
            100
                               105
 Val Ser Asp Val Ala Glu Ala Pro Lys Ser Ala Ser Glu Arg Ala Ala
                            120
 Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His Tyr Pro
                        135
                                           140
Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys Asn Ala
                                       155
Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu
                165
                                   170
Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val His
Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu
                           200
                                              205
Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys Asp
                       215
Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp Ile
                   230
                                      235
Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln
               245
                                  250
Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser
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			260)				265	;				270)	
Phe	e Val	G1r 279		∨a1	Cys	Asn	G1u 280		Leu	Thr	His	7rp 285		e G1u	ı Ala
Asr	290		^ Asp	Ser	Lys	Va1 295		۷a٦	Asn	Lys			l Ser	- Ser	- Ala
α.			, T],	. 47-	47.			47.	A		300		1 4		
305					310	1				31.5	i				320
Ser	· Ala	Thr	· Asp	325		Gly	Leu	Pro	GTy 330		Leu	ı Ala	a Asp	335 335	
Ser	Thr	Asp	Pro 340		l Lys	Ser	Glu	Leu 345		Val	Val	GTu	ւ GTy 350		Ser
ΑΊa	G7y	GTy 355		· Ala	Lys	Ser	GTy 360		Asp	Ser	Met	: Phe	G]n		Ile
Leu	Pro 370	Leu		GTy	Lys		Ile		Val	Glu		Ala		ı Ile	· Asp
	Va1		Lys	Asn	Thr	375 Glu		Gln	Ala	Ile	380 Ile		Ala	Leu	СТy
385					390					395					400
Thr	Gly	Ile	His	Asp 405		Phe	Asp	Ile	GTy 410		Leu	Arg	Tyr	His 415	
Πe	Val	Leu													
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	0> 3:														
Ser	Asp	Ala	Tyr	Ala	Ile	Ser	Gly	Gly	Leu	His	Gly	Val	Gly	Val	Ser
1				5					10					15	
Val	Val	Asn		Leu	Ser	Thr	Arg	Leu	Glu	Va1	Glu	IJе	Lys	Arg	Asp
	_		20					25					30		
Gly	Tyr			Ser	Gln	Val		Glu	Lys	Ser	Glu		Leu	Gly	Leu
		35		_	_		40					45			
Lys	Gln	Gly	Ala	Pro	Thr		Lys	Thr	Gly	Ser		Val	Arg	Phe	Trp
	50				_	55		_		_	60				_
	Asp	Pro	Ala	Val		Glu	Thr	Thr	Glu			Phe	Glu	Thr	
65		.		<i>6</i> 7	70		47.	~		. 75				_	80
АІа	Arg	Arg	Leu	GIn 85	Glu	Met	Ala	Phe	Leu 90	Asn	Lys	Gly	Leu	Thr 95	IJe
Asn	Leu	Thr	Asp 100	Glu	Arg	Val		G]n 105	Asp	Glu	Val	Val	Asp 110	Glu	۷a٦
Val	Ser	Asp		Ala	Glu	Ala			Ser	Ala	Ser	Glu		Δla	Δla
		115		,		,,,,	120	_,_	50,	,u	٥.,	125	Ai g	Ala	Λια
Glu	Ser		Ala	Pro	His	lvs		Lvs	Ser	Ara	Thr		His	Tvr	Pm
	130	••••	,,, <u>u</u>		5	135	,	L 7 <i>3</i>	501	AI 9	140	1116	1113	1 9 1	rio
Glv	Gly	Leu	Val	Asp	Phe		Lvs	His	Tle	Asn		Thr	Lvs	Δsn	Δla
145	,			,	150		-,5			155	/ 11 SI		LyJ	7011	160
	His	Ser	Ser	Ile		Asp	Phe	Ser	Glv		Glv	Thr	GTV	Hic	
. 2				165	••				170	_,_	,		,	175	4
۷a٦	Glu	Ile	Ala		Gln	Tmo	Asn			Tvr	Ser	Glu	Ser		His
			180			•		185	•			_ , _	190		
Thr	Phe	Ala		Thr	Ile	Asn			Glu	σīγ	GJV	Thr		Glu	Glu

195 200 205	
Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys A	۸cn
210 215 220	ъ
Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp :	
225 220 225	
233	240
Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro (Jlu
245 250 255	
Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys S	er
260 265 270	
Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu A	ıî a
275 280 285	ιια
Asn Pro Thr Asp Ala Lys Val Val Val Asn Lys Ala Val Ser Ser A	-
200	ııа
233 300	
Gin Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg L	ys
305 310 315 3	20
Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys A	rg
325 330 ₃₃₅	
Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp S	or
340 345 350	-1
Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala I	
355	ıe
200	
Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile As	s p
370 375 380	
Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gl	y
385 390 395 40	
Thr Gly Ile His Asp Glu Phe Asp Ile Gly Lys Leu Arg Tyr His Ly	/ S
405 410 415	-
Ile Val Leu	
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<211> 419	
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·	
<213> Mycobacterium gastri	
<400> 32	
Ser Asp Ala Tyr Ala Ile Ser Gly Gly Leu His Gly Val Gly Val Se	r
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Val Val Asn Ala Leu Ser Ile Arg Leu Glu Val Glu Ile Lys Arg Asp	n
20 25 . 30	,
Gly His Glu Trp Ser Gln Val Tyr Glu Lys Ser Glu Pro Met Gly Leu	
25	1
43	
Lys Gln Gly Ala Pro Thr Lys Lys Thr Gly Thr Thr Val Arg Phe Tr)
50 55 60	
Ala Asp Pro Asn Val Phe Glu Thr Thr Glu Tyr Asp Phe Glu Thr Val	ļ
65 70 75 80	
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30 93	
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100 105 110	
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115 120 125	
Glu Phe Thr Ala Pro His Lys Val Lys Lys Arg Thr Phe His Tyr Pro	

· 92

135

Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys Asn Ala 150 155

Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu 165 170

Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val His 185

Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu 200

Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys Asp

Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp Ile 230 235

Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln 250

Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser 265

Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala 280

Asn Pro Ala Asp Ala Lys Thr Val Val Asn Lys Ala Val Ser Ser Ala 295 300

Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys

Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg 330

Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser

Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile 360

Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp 375

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<212> PRT

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Arg Asp Gly Tyr Glu Trp Ser Gln Phe Tyr Asp Arg Ala Gln Pro Gly 40

Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg

Phe Trp Ala Asp Ser Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu

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                                     170
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                                        235
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 Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
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Ser Ala Gln Ala Arg Leu Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
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Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
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Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
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                                                       415
His Lys Ile Val Leu
           420
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<211> 419
<212> PRT
<213> Mycobacterium microti
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IJγ	Tyr	G1u 35		Ser	Gln	Val	Tyr 40		Lys	Ser	Glu	Pro		Gly	Leu
Lys	G1n 50	Gly		Pro	Thr		Lys		· GTy	Ser				Phe	Trp
AΊα			47.	Val	Dha	55		TL -	٠.٠	T	60	~	63.	_	
65		Pro	на	vai	70	Giu	inr	inr	Giu	75	ASp	me	Giu	ınr	Va I 80
Αla	Arg	Arg	Leu	Gln	Glu	Met	Αla	Phe	Leu	Asn	Lvs	GTy	Leu	Thr	
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ASN	Leu	Thr	100	Giu	Arg	vai	Inr	105		Glu	Val	Vai	Asp 110	Glu	Va1
Val	Ser	Asp 115	Val	Αla	Glu	Ala	Pro 120	Lys	Ser	Ala	Ser	Glu 125	Arg	Αla	Ala
Glu	Ser 130	Thr	Аlа	Pro	His	Lys 135	۷a٦	Lys	Ser	Arg	Thr 140	Phe	His	Tyr	Pro
۵lv		Leu	Va1	Δsn	Phe		Lvc	Hic	Tla	Δcn		The	Lve	Acn	Αla
145	,			. – բ	150		~,5			155	/ U 54	••••	LyJ	ASII	160
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			180					185					190		
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σīν	Phe	Arg		Ala	Leu	Thr		Val	Val	Asn	Ivs		Δla	Lvs	Δsn
·	210	.•				215					220	.,.	, <u></u>	-,5	, ор
Arg	Lys	Leu	Leu	Lys	Asp	Lys	Asp	Pro	Asn	Leu	Thr	Gly	Asp	Asp	Ile
225					230					235					240
Arg	Glu	Gly		A1a 245	Ala	Val	Ile	Ser	Va1 250	Lys	Val	Ser	Glu	Pro 255	ឲា
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Phe	Val	G1n 275	Lys	Val	Cys	Asn	G]u 280	Gln	Leu	Thr		Trp 285	Phe	Glu	Ala
	Pro 290	Thr	Asp	Ser		Va1 295	Val	Val	Asn	Lys	A1a 300	۷a٦	Ser	Ser	Ala
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Ser	Ala	Thr			IJУ	G٦y	Leu	Pro		Lys	Leu	ΑΊa	Asp		
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			340					345					350		
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	Pro 370	Leu .	Arg	G]y		Ile 375	Ile .	Asn	Val		Lys . 380	Ala .	Arg	Ile	Asp
		Leu	Lvs	Asn '			Val :	նշո	Δla			Thr	Δla	Leu	ДV
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Lys Asp Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp
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                                        235
Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ala Glu
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Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
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Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
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                                    330
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                                    170
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                                           220
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                                          315
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                        295
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                Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
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                                345
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His Lys Ile Val Leu
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【図面の簡単な説明】

*を用いたPCRの結果を示す図。

【図1】マイコバクテリウム・カンサシイとマイコバク テリウム・ガストリのgyrB遺伝子の塩基配列を示す図。

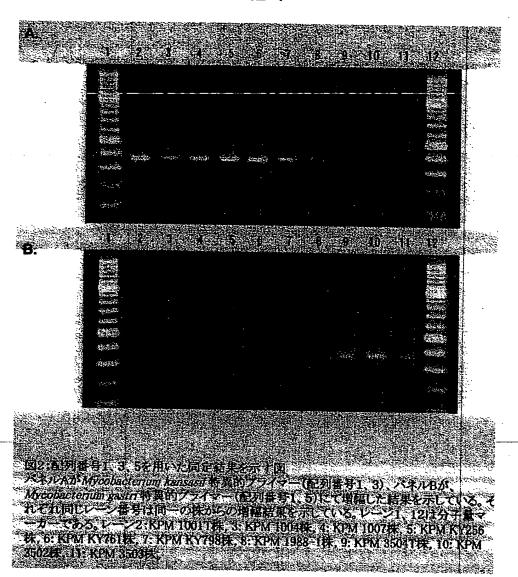
【図3】分子系統解析により作製された遅発育性マイコ バクテリアの系統樹を示す図。

【図2】配列番号1、3、5により表されるプライマー*

【図1】

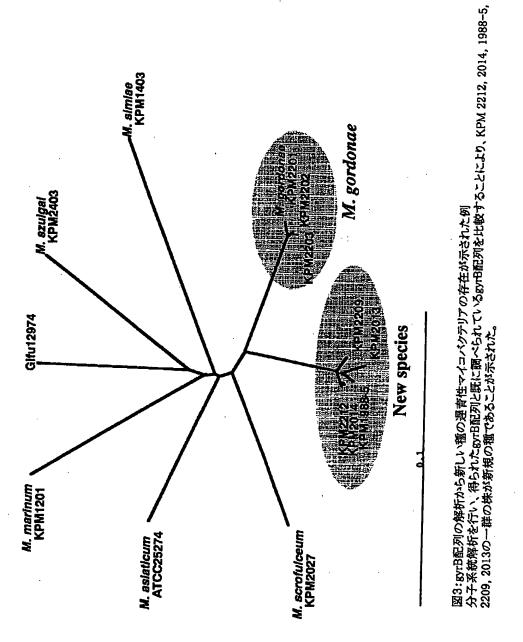
MGAS	
MEAN NGAS	
HEAR	
War Was	
ngan Ngas	
WAN Was	201:
WEAR NGAS	361:
MICAS MGAS	421:TTGT
MEAS	480: CATOCACACCACOTOCHCHICTOCOCTALOGGACCOSOCCACGAAGTICACATOG 528 480:
MGAS	S39: CEARCCASTGCAATGCCCACTATCCGAGTGCGTGCATACCTTGGCCAACACCATCAACA 598 S39: CEARCCASTGCAATGCCCACTCACCATCACCATCAACA 598 S99: CEARCCASTGCAATGCCCACTCACCATCACCATCAACA 598 S99: CEARCCASTGCAATGCCCACTCACCATCACCATCACCATCACCACCACCACCACC
YXAN NGAS	E99:00CAGCAGGGTGGGAOCCAGGAGAGGGGTTTOGCGGGGCCCCACUTOGCTGGTGAACA 658 699:
NGAS	659: ACTADOCCARGACOCCAACTGCTCARGGLAAGCACCTCARCTCACCTCACACACACACACACACACACACAC
MEAN MGAS	719: TOCKGEAMGGETTGGOGGGETCATTTGGTTCAMGGTCAGGGGGCAGTTUGAGGGGC 778 719:
NGAS	779:
NGAS	899 : 005171CARCECCEAGCACCCATTGCGGCCCCAGCGCGCGCGCGCGCGCGCGCG
NGAS	899:.A
NGAS NGAS	958:AAGAGGGCAGCGATCTGGCCGAGCTGCCCAGCTGCCCGACTGCCGCGACTGCGCGACTGCACTGCCGCAGCTGCACTGCCGCAGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG
NGAS	1018:C.TT.A
IIGAS	1078:-C. T. A. C. 1137
NGAS	1138: AMGGOODGCA FOLACODGGROCTEANGAGCHOCAMETOCAGGOGAFICATCACOGGOTTE 1197 1138:
IIGAS	1198:C
图1: 同一の	Bycobacterium kansssii(上限)と Nycobacterium gastri(下間)の gyr6 配列の比較。 機基配列の部分は変印で示し、Nycobacterium kansasii の配列のみを示した。

【図2】



)

【図3】



フロントページの続き

(72)発明者 原山 重明

岩手県釜石市平田第3地割75番1 株式会 社海洋バイオテクノロジー研究所釜石研究 所内 F ターム(参考) 48024 AA11 CA09 DA05 HA19 48063 QA01 QQ06 QQ42 QR32 QR55 QR62 QS25

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The 16s/23s ribosomal spacer region as a target for DNA probes to identify eubacteria.

Barry T, Colleran G, Glennon M, Dunican LK, Gannon F.

National Diagnostic Centre, BioResearch Ireland, University College Galway.

Variable regions of the 16s ribosomal RNA have been frequently used as the target for DNA probes to identify microorganisms. In some situations, however, there is very little sequence variation observed between the 16s rRNA genes of closely related microorganisms. This study presents a general method to obtain species-specific probes using the spacer (intergenic) region between the 16s and 23s rRNA genes. The overall strategy is analogous to that which has previously been developed for the variable regions of the 16s rRNA genes. Sequence analysis of the 16s rRNA and 23s rRNA coding sequences flanking the spacer regions resulted in the design of PCR primers that can be used to amplify the spacer regions of a wide range of eubacteria. Sequencing the amplified spacer region then gives rise to the information that can be used to select specific DNA sequences for use as a DNA probe or for the generation of specific PCR primers to a microorganism of interest. In this study the approach to develop specific DNA markers for members of the genus Clostridium is described in detail. A specific DNA oligonucleotide probe and PCR primers have been designed for Clostridium perfringens that distinguish it from other organisms in the genus.

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